```
  * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 09:24:45 ON 26 AUG 2003

=> fil .bec
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       1.26       1.26

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
      ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 09:28:10 ON 26 AUG 2003
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s bacillus thuringiensis
FILE 'MEDLINE'
         43510 BACILLUS
          2918 THURINGIENSIS
L1        2821 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

FILE 'SCISEARCH'
         43100 BACILLUS
          5566 THURINGIENSIS
L2        5253 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

FILE 'LIFESCI'
         23286 "BACILLUS"
          3921 "THURINGIENSIS"
L3        3853 BACILLUS THURINGIENSIS
                 ("BACILLUS"(W)"THURINGIENSIS")

FILE 'BIOTECHDS'
         15549 BACILLUS
          2169 THURINGIENSIS
L4        2157 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

FILE 'BIOSIS'
         62854 BACILLUS
          8618 THURINGIENSIS
L5        8547 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

FILE 'EMBASE'
         32058 "BACILLUS"
          2211 "THURINGIENSIS"
L6        2162 BACILLUS THURINGIENSIS
                 ("BACILLUS"(W)"THURINGIENSIS")

FILE 'HCAPLUS'
         75892 BACILLUS
          5915 THURINGIENSIS
L7        5793 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

FILE 'NTIS'
          1623 BACILLUS
           183 THURINGIENSIS
L8         168 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)
```

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FILE 'ESBIOBASE'
         12487 BACILLUS
          1722 THURINGIENSIS
L9        1686 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

FILE 'BIOTECHNO'
         19348 BACILLUS
          2216 THURINGIENSIS
L10       2184 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

FILE 'WPIDS'
         10820 BACILLUS
           980 THURINGIENSIS
L11        887 BACILLUS THURINGIENSIS
                 (BACILLUS(W)THURINGIENSIS)

TOTAL FOR ALL FILES
L12      35511 BACILLUS THURINGIENSIS

=> s (truncat? or digest? or fragment?)(4a)(endotoxin# or toxin# or crystal
protein#)
FILE 'MEDLINE'
        156202 TRUNCAT?
        102522 DIGEST?
        246783 FRAGMENT?
         27257 ENDOTOXIN#
         70410 TOXIN#
         36322 CRYSTAL
       1524628 PROTEIN#
          1150 CRYSTAL PROTEIN#
                 (CRYSTAL(W)PROTEIN#)
L13       1251 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
               CRYSTAL PROTEIN#)

FILE 'SCISEARCH'
         38255 TRUNCAT?
         83325 DIGEST?
        201235 FRAGMENT?
         27149 ENDOTOXIN#
         62612 TOXIN#
        350618 CRYSTAL
       1213753 PROTEIN#
          1003 CRYSTAL PROTEIN#
                 (CRYSTAL(W)PROTEIN#)
L14        999 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
               CRYSTAL PROTEIN#)

FILE 'LIFESCI'
         14110 TRUNCAT?
         33309 DIGEST?
         83011 FRAGMENT?
          6902 ENDOTOXIN#
         31186 TOXIN#
         13485 "CRYSTAL"
        468458 PROTEIN#
           444 CRYSTAL PROTEIN#
                 ("CRYSTAL"(W)PROTEIN#)
L15        692 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
               CRYSTAL PROTEIN#)

FILE 'BIOTECHDS'
          2460 TRUNCAT?
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         15040 DIGEST?
         39927 FRAGMENT?
           977 ENDOTOXIN#
          4846 TOXIN#
          2953 CRYSTAL
        111402 PROTEIN#
          1551 CRYSTAL PROTEIN#
                 (CRYSTAL(W)PROTEIN#)
L16          392 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
                 CRYSTAL PROTEIN#)

FILE 'BIOSIS'
         32305 TRUNCAT?
        543740 DIGEST?
        212724 FRAGMENT?
         24608 ENDOTOXIN#
        147650 TOXIN#
         42662 CRYSTAL
       1519880 PROTEIN#
           779 CRYSTAL PROTEIN#
                 (CRYSTAL(W)PROTEIN#)
L17         1617 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
                 CRYSTAL PROTEIN#)

FILE 'EMBASE'
         22589 TRUNCAT?
        139579 DIGEST?
        157580 FRAGMENT?
         23284 ENDOTOXIN#
         62018 TOXIN#
         44818 "CRYSTAL"
       1201342 PROTEIN#
           307 CRYSTAL PROTEIN#
                 ("CRYSTAL"(W)PROTEIN#)
L18         1010 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
                 CRYSTAL PROTEIN#)

FILE 'HCAPLUS'
         37362 TRUNCAT?
        211401 DIGEST?
        340917 FRAGMENT?
         25586 ENDOTOXIN#
        101730 TOXIN#
       1047908 CRYSTAL
       1783258 PROTEIN#
          1389 CRYSTAL PROTEIN#
                 (CRYSTAL(W)PROTEIN#)
L19         1882 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
                 CRYSTAL PROTEIN#)

FILE 'NTIS'
          3383 TRUNCAT?
          5888 DIGEST?
         13082 FRAGMENT?
           721 ENDOTOXIN#
          3293 TOXIN#
         41763 CRYSTAL
         17056 PROTEIN#
             7 CRYSTAL PROTEIN#
                 (CRYSTAL(W)PROTEIN#)
L20           27 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
                 CRYSTAL PROTEIN#)

FILE 'ESBIOBASE'
```

```
         16421 TRUNCAT?
         38859 DIGEST?
         68158 FRAGMENT?
          5691 ENDOTOXIN#
         25220 TOXIN#
         23012 CRYSTAL
        541573 PROTEIN#
           210 CRYSTAL PROTEIN#
                 (CRYSTAL(W)PROTEIN#)
L21        430 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
               CRYSTAL PROTEIN#)

FILE 'BIOTECHNO'
         17873 TRUNCAT?
         39804 DIGEST?
        101728 FRAGMENT?
          5446 ENDOTOXIN#
         24136 TOXIN#
         14868 CRYSTAL
        623951 PROTEIN#
           305 CRYSTAL PROTEIN#
                 (CRYSTAL(W)PROTEIN#)
L22        630 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
               CRYSTAL PROTEIN#)

FILE 'WPIDS'
         25007 TRUNCAT?
         20486 DIGEST?
         56369 FRAGMENT?
          2183 ENDOTOXIN#
          6849 TOXIN#
        242339 CRYSTAL
        114762 PROTEIN#
           183 CRYSTAL PROTEIN#
                 (CRYSTAL(W)PROTEIN#)
L23        325 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
               CRYSTAL PROTEIN#)

TOTAL FOR ALL FILES
L24       9255 (TRUNCAT? OR DIGEST? OR FRAGMENT?)(4A)(ENDOTOXIN# OR TOXIN# OR
               CRYSTAL PROTEIN#)

=> s l12 and l24
FILE 'MEDLINE'
L25         56 L1 AND L13

FILE 'SCISEARCH'
L26         70 L2 AND L14

FILE 'LIFESCI'
L27         79 L3 AND L15

FILE 'BIOTECHDS'
L28        113 L4 AND L16

FILE 'BIOSIS'
L29        103 L5 AND L17

FILE 'EMBASE'
L30         47 L6 AND L18

FILE 'HCAPLUS'
L31        179 L7 AND L19
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FILE 'NTIS'
L32          0 L8 AND L20

FILE 'ESBIOBASE'
L33         31 L9 AND L21

FILE 'BIOTECHNO'
L34         49 L10 AND L22

FILE 'WPIDS'
L35         33 L11 AND L23

TOTAL FOR ALL FILES
L36        760 L12 AND L24

=> s l12(15a)l24
FILE 'MEDLINE'
L37         23 L1 (15A)L13

FILE 'SCISEARCH'
L38         32 L2 (15A)L14

FILE 'LIFESCI'
L39         35 L3 (15A)L15

FILE 'BIOTECHDS'
L40         63 L4 (15A)L16

FILE 'BIOSIS'
L41         40 L5 (15A)L17

FILE 'EMBASE'
L42         17 L6 (15A)L18

FILE 'HCAPLUS'
L43         88 L7 (15A)L19

FILE 'NTIS'
L44          0 L8 (15A)L20

FILE 'ESBIOBASE'
L45         10 L9 (15A)L21

FILE 'BIOTECHNO'
L46         20 L10(15A)L22

FILE 'WPIDS'
L47         18 L11(15A)L23

TOTAL FOR ALL FILES
L48        346 L12(15A) L24

=> s cryvi? or cry6? or 86a1 or ps86a1
FILE 'MEDLINE'
             0 CRYVI?
             2 CRY6?
             0 86A1
             0 PS86A1
L49          2 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'SCISEARCH'
             0 CRYVI?
             3 CRY6?
             1 86A1
```

```
              0 PS86A1
L50           4 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'LIFESCI'
              0 CRYVI?
              3 CRY6?
              1 86A1
              3 PS86A1
L51           7 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'BIOTECHDS'
              2 CRYVI?
              2 CRY6?
              4 86A1
              6 PS86A1
L52          12 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'BIOSIS'
              0 CRYVI?
              8 CRY6?
              5 86A1
              0 PS86A1
L53          13 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'EMBASE'
              0 CRYVI?
              1 CRY6?
              0 86A1
              0 PS86A1
L54           1 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'HCAPLUS'
              5 CRYVI?
             12 CRY6?
              4 86A1
              3 PS86A1
L55          22 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'NTIS'
              0 CRYVI?
              0 CRY6?
              0 86A1
              0 PS86A1
L56           0 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'ESBIOBASE'
              0 CRYVI?
              0 CRY6?
              0 86A1
              0 PS86A1
L57           0 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'BIOTECHNO'
              0 CRYVI?
              1 CRY6?
              0 86A1
              0 PS86A1
L58           1 CRYVI? OR CRY6? OR 86A1 OR PS86A1

FILE 'WPIDS'
              1 CRYVI?
              6 CRY6?
              3 86A1
              6 PS86A1
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L59          14 CRYVI? OR CRY6? OR 86A1 OR PS86A1

TOTAL FOR ALL FILES
L60          76 CRYVI? OR CRY6? OR 86A1 OR PS86A1

=> s (l48 or l60) not 1999-2003/py
FILE 'MEDLINE'
       2313778 1999-2003/PY
L61          22 (L37 OR L49) NOT 1999-2003/PY

FILE 'SCISEARCH'
       4488524 1999-2003/PY
L62          28 (L38 OR L50) NOT 1999-2003/PY

FILE 'LIFESCI'
        467385 1999-2003/PY
L63          33 (L39 OR L51) NOT 1999-2003/PY

FILE 'BIOTECHDS'
         81530 1999-2003/PY
L64          64 (L40 OR L52) NOT 1999-2003/PY

FILE 'BIOSIS'
       2455479 1999-2003/PY
L65          37 (L41 OR L53) NOT 1999-2003/PY

FILE 'EMBASE'
       2032597 1999-2003/PY
L66          14 (L42 OR L54) NOT 1999-2003/PY

FILE 'HCAPLUS'
       4326642 1999-2003/PY
L67          62 (L43 OR L55) NOT 1999-2003/PY

FILE 'NTIS'
         81635 1999-2003/PY
L68           0 (L44 OR L56) NOT 1999-2003/PY

FILE 'ESBIOBASE'
       1308920 1999-2003/PY
L69           5 (L45 OR L57) NOT 1999-2003/PY

FILE 'BIOTECHNO'
        556086 1999-2003/PY
L70          15 (L46 OR L58) NOT 1999-2003/PY

FILE 'WPIDS'
       3816663 1999-2003/PY
L71          11 (L47 OR L59) NOT 1999-2003/PY

TOTAL FOR ALL FILES
L72         291 (L48 OR L60) NOT 1999-2003/PY

=> log y
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY    SESSION
FULL ESTIMATED COST                              22.27      23.53

STN INTERNATIONAL LOGOFF AT 09:36:19 ON 26 AUG 2003
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	L #	Hits	Search Text	DBs	Time Stamp
1	L1	3211	BACILLUS ADJ THURINGIENSIS	USPAT; US-PGPUB	2003/08/26 09:00
2	L2	94	CRYVI$2 OR CRY6$2	USPAT; US-PGPUB	2003/08/26 09:01
3	L3	3266	((TRUNCAT$6 OR DIGEST$6 OR FRAGMENT$6) NEAR3 (TOXIN$1 OR (CRYSTAL ADJ PROTEIN$1)))	USPAT; US-PGPUB	2003/08/26 09:01
4	L4	56	86A1 OR PS86A1	USPAT; US-PGPUB	2003/08/26 09:02
5	L5	126	1 same 3	USPAT; US-PGPUB	2003/08/26 09:02
6	L6	56	(2 or 4) and 3	USPAT; US-PGPUB	2003/08/26 09:02
7	L7	163	5 or 6	USPAT; US-PGPUB	2003/08/26 09:02

PGPUB-DOCUMENT-NUMBER: 20030119158

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030119158 A1

TITLE: Polynucleotide compositions encoding broad-spectrum delta endotoxins

PUBLICATION-DATE: June 26, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Malvar, Thomas	Dublin	PA	US	
Gilmer, Amy Jelen	Langhorne	PA	US	

APPL-NO: 09/ 997914

DATE FILED: November 30, 2001

RELATED-US-APPL-DATA:

child 09997914 A1 20011130

parent division-of 09261040 19990302 US PATENTED

child 09261040 19990302 US

parent division-of 08754490 19961120 US PATENTED

US-CL-CURRENT: 435/184, 435/252.3 , 435/69.2 , 536/23.7

ABSTRACT:

Disclosed are novel synthetically-modified B. thuringiensis chimeric crystal proteins having improved insecticidal activity against coleopteran, dipteran and lepidopteran insects. Also disclosed are the nucleic acid segments encoding these novel peptides. Methods of making and using these genes and proteins are disclosed as well as methods for the recombinant expression, and transformation of suitable host cells. Transformed host cells and transgenic plants expressing the modified endotoxin are also aspects of the invention.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (40):

[0038] Favorable traits with regard to improved insecticidal activity, increased host range, and improved production characteristics may be achieved by other such hybrid .delta.-endotoxins including, but not limited to, the cry1, cry2, cry3, cry4, cry5, **cry6,** cry7, cry8, cry9, cry10, cry11, cry12,

cry13, cry14, cry15 class of .delta.-endotoxin genes and the B. thuringiensis cytolytic cyt1 and cyt2 genes. Members of these classes of B. thuringiensis insecticidal proteins include, but are not limited to Cry1Aa, Cry1Ab, Cry1Ac , Cry1Ad, Cry1Ae, Cry1Ba, Cry1Bb, Cry1Ca, Cry1Cb, Cry1Da, Cry1Db, Cry1Ea, Cry1Eb, Cry1Fa, Cry1Fb, Cry1Ga, Cry1Ha, Cry2a, Cry2b, Cry1Ja, Cry1Ka, Cry11Aa, Cry11Ab, Cry12Aa, Cry3Ba, Cry3Bb, Cry3C, Cry4a, Cry4Ba, Cry5a, Cry5Ab, **Cry6Aa, Cry6Ba,** Cry7Aa, Cry7Ab, Cry8Aa, Cry8Ba, Cry8Ca, Cry9Aa, Cry9Ba, Cry9Ca, Cry10Aa, Cry11Aa, Cry12Aa, Cry13Aa, Cry14Aa, Cry15Aa, Cyt1Aa, and Cyt2Aa. Related hybrid .delta.-endotoxins would consist of the amino portion of one of the aforementioned .delta.-endotoxins, including all or part of domain 1 or domain 2, fused to all or part of domain 3 from another of the aforementioned .delta.-endotoxins. The non-active protoxin fragment of such hybrid .delta.-endotoxins may consist of the protoxin fragment from any of the aforementioned .delta.-endotoxins which may act to stabilize the hybrid .delta.-endotoxin as demonstrated by EG11087 and EG11091 (see e.g., TABLE 3). Hybrid .delta.-endotoxins possessing similar traits as those described in the present invention could be constructed by conservative, or "similar" replacements of amino acids within hybrid .delta.-endotoxins. Such substitutions would mimic the biochemical and biophysical properties of the native amino acid at any position in the protein. Amino acids considered similar include for example, but are not limited to:

Summary of Invention Paragraph - BSTX (47):

[0045] Researchers skilled in the art will recognize that improved insecticidal activity, increased host range, and improved production characteristics imparted upon hybrid .delta.-endotoxins may be further improved by altering the genetic code for one or more amino acid positions in the hybrid .delta.-endotoxin such that the position, or positions, is replaced by any other amino acid. This may be accomplished by targeting a region or regions of the protein for mutagenesis by any number of established mutagenic techniques, including those procedures relevant to the present invention. Such techniques include site-specific mutagenesis (Kunkle, 1985; Kunkle et al., 1987), DNA shuffling (Stemmer, 1994), and PCR.TM. overlap extension (Horton et al., 1989). Since amino acids situated at or near the surface of a protein are likely responsible for its interaction with other proteinaceous or non-proteinaceous moieties, they may serve as "target" regions for mutagenesis. Such surface exposed regions may consist of, but not be limited to, surface exposed amino acid residues within the active **toxin fragment** of the protein and include the inter-.alpha.-helical or inter-.beta.-strand "loop" -regions of .delta.-endotoxins that separate .alpha.-helices within domain 1 and .beta.-strands within domain 2 and domain 3. Such procedures may favorably change the protein's biochemical and biophysical characteristics or its mode of action as outlined in the Section 1. These include, but are not limited to: 1) improved crystal formation, 2) improved protein stability or reduced protease degradation, 3) improved insect membrane receptor recognition and binding, 4) improved oligomerization or channel formation in the insect midgut endothelium, and 5) improved insecticidal activity or insecticidal specificity due to any or all of the reasons stated above.

Summary of Invention - Table CWU - BSTL (1):

1TABLE 1 Revised B. thuringiensis .delta.-Endotoxin Nomenclature.sup.a

New Old GenBank Accession # Cry1Aa CryIA(a) M11250 Cry1Ab CryIA(b) M13898 Cry1Ac CryIA(c) M11068 Cry1Ad CryIA(d) M73250 Cry1Ae CryIA(e) M65252 Cry1Ba CryIB X06711 Cry1Bb ET5 L32020 Cry1Bc PEG5 Z46442 Cry1Ca CryIC X07518 Cry1Cb CryIC(b) M97880 Cry1Da CryID X54160 Cry1Db PrtB Z22511 Cry1Ea CryIE X53985 Cry1Eb CryIE(b) M73253 Cry1Fa CryIF M63897 Cry1Fb PrtD Z22512 Cry1G PrtA Z22510 Cry1H PrtC Z22513 Cry1Hb U35780 Cry2a CryV X62821 Cry2b CryV U07642 Cry2Ja ET4 L32019 Cry1Jb ET1 U31527 Cry1K U28801 Cry2Aa CryIIA M31738 Cry2Ab CryIIB M23724 Cry2Ac CryIIC X57252 Cry3A CryIIIA M22472 Cry3Ba CryIIIB X17123 Cry3Bb CryIIIB2 M89794 Cry3C CryIIID X59797 Cry4A CryIVA Y00423 Cry4B CryIVB X07423 Cry5Aa CryVA(a) L07025 Cry5Ab CryVA(b) L07026 Cry5B U19725 **Cry6A CryVIA** L07022 **Cry6B CryVIB** L07024 Cry7Aa CryIIIC M64478 Cry7Ab CryIIICb U04367 Cry8A CryIIIE U04364 Cry8B CryIIIG U04365 Cry8C CryIIIF U04366 Cry9A CryIG X58120 Cry9B CryIX X75019 Cry9C CryIH Z37527 Cry10A CryIVC M12662 Cry11A CryIVD M31737 Cry11B Jeg80 X86902 Cry12A CryVB L07027 Cry13A CryVC L07023 Cry14A CryVD U13955 Cry15A 34kDa M76442 Cry16A cbm71 X94146 Cyt1A CytA X03182 Cyt2A CytB Z14147 .sup.aAdapted from: http://epunix.biols.susx.ac.uk/Home/Ne- il_Crickmore/Bt/index.html

Detail Description Paragraph - DETX (137):

[0302] The majority of hybrids involving Cry1Ac and Cry1F formed stable crystals in B. thuringiensis A notable exception is EG11088 in which the active **toxin fragment** would be the reciprocal exchange of EG11063. Two of the three hybrids involving Cry1Ac and Cry1C, EG11087 and EG11090, failed to produce crystal in B. thuringiensis even though these reciprocal hybrids mimic the activated **toxin fragments** of crystal-forming EG11063 and EG11074.

Detail Description Paragraph - DETX (141):

[0304] Proteolytic degradation of the protoxin form of the .delta.-endotoxin to a stable active toxin occurs once .delta.-endotoxin crystals are solubilized in the larval midgut. One measure of the potential activity of .delta.-endotoxins is the stability of the active .delta.-endotoxin in a proteolytic environment. To test the proteolytic sensitivity of the hybrid .delta.-endotoxins, solubilized **toxin was subjected to trypsin digestion**. The .delta.-endotoxins were purified from sporulated B. thuringiensis cultures and quantified as described by Chambers et al., 1991. Exactly 250 .mu.g of each hybrid .delta.-endotoxin crystal was solubilized in 30 mM NaHCO.sub.3, 10 mM DTT (total volume 0.5 ml). Trypsin was added to the solubilized toxin at a 1:10 ratio. At appropriate time points 50 .mu.l aliquots were removed to 50 .mu.l Laemmli buffer, heated to 100.degree. C. for 3 min., and frozen in a dry-ice ethanol bath for subsequent analysis. The trypsin **digests of the solubilized toxins** were analyzed by SDS-PAGE and the amount of active .delta.-endotoxin at each time point was quantified by densitometry. A graphic representation of the results from these studies are shown in FIG. 3.

Detail Description Paragraph - DETX (149):

[0310] The .delta.-endotoxins described in FIG. 1 and that demonstrated insecticidal activity in bioassay screens were tested as purified crystals to determine their LC.sub.50 (see TABLE 5). The .delta.-endotoxins purified from strains EG11063, EG11074, EG11091, and EG11735 all show increased armyworm (S. frugiperda and S. exigua) activity compared to any of the wild-type

.delta.-endotoxins tested. The EG11063 and EG11074 .delta.-endotoxins would yield identical active **toxin fragments** (refer to FIG. 1B) which is evident by their similar LC50 values on the insects examined. An unexpected result evident from these data is that a hybrid .delta.-endotoxin such as EG11063, EG11092, EG11074, EG11735, or EG11751 can retain the activity of their respective parental .delta.-endotoxins, and, against certain insects such as S. exigua, can have activity far better than either parental .delta.-endotoxin. This broad range of insecticidal activity at doses close to or lower than the parental .delta.-endotoxins, along with the wild-type level of toxin production (see Example 2), make these proteins particularly suitable for production in B. thuringiensis. Although the EG11091 derived .delta.-endotoxin has better activity against S. frugiperda and S. exigua than its parental .delta.-endotoxins, it has lost the H. virescens and H. zea activity attributable to its Cry1Ac parent. This restricted host range along with lower toxin yield observed for the EG11091 .delta.-endotoxin (see Example 2) make it less amenable to production in B. thuringiensis

Detail Description Paragraph - DETX (322):

[0477] The majority of hybrids involving Cry1Ac and Cry1F formed stable crystals in B. thuringiensis A notable exception is EG11088 in which the active **toxin fragment** would be the reciprocal exchange of EG11063. Two of the three hybrids involving Cry1Ac and Cry1C, EG11087 and EG11090, failed to produce crystal in B. thuringiensis even though these reciprocal hybrids mimic the activated **toxin fragments** of crystal-forming EG11063 and EG11074.

Detail Description Paragraph - DETX (326):

[0479] Proteolytic degradation of the protoxin form of the .delta.-endotoxin to a stable active toxin occurs once .delta.-endotoxin crystals are solubilized in the larval midgut. One measure of the potential activity of .delta.-endotoxins is the stability of the active .delta.-endotoxin in a proteolytic environment. To test the proteolytic sensitivity of the hybrid .delta.-endotoxins, solubilized **toxin was subjected to trypsin digestion**. The .delta.-endotoxins were purified from sporulated B. thuringiensis cultures and quantified as described by Chambers et al., 1991. Exactly 250 .mu.g of each hybrid .delta.-endotoxin crystal was solubilized in 30 mM NaHCO.sub.3, 10 mM DTT (total volume 0.5 ml). Trypsin was added to the solubilized toxin at a 1:10 ratio. At appropriate time points 50 .mu.l aliquots were removed to 50 .mu.l Laenunli buffer, heated to 100.degree. C. for 3 min., and frozen in a dry-ice ethanol bath for subsequent analysis. The trypsin **digests of the solubilized toxins** were analyzed by SDS-PAGE and the amount of active .delta.-endotoxin at each time point was quantified by densitometry. A graphic representation of the results from these studies are shown in FIG. 3.

PGPUB-DOCUMENT-NUMBER: 20030115628

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030115628 A1

TITLE: Nucleotide sequences coding for polypeptides endowed with a larvicidal activity towards lepidoptera

PUBLICATION-DATE: June 19, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Vincent, Sanchis	Cambridge		GB	
Didier, Lereclus	Paris		FR	
Ghislaine, Menou	Paris		FR	
Marguerite-Marie, Lecadet	Paris		FR	
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APPL-NO: 09/ 918485

DATE FILED: August 1, 2001

RELATED-US-APPL-DATA:

child 09918485 A1 20010801

parent division-of 09037621 19980310 US GRANTED

parent-patent 6310035 US

child 09037621 19980310 US

parent division-of 08461551 19950605 US GRANTED

parent-patent 5792928 US

child 08461551 19950605 US

parent division-of 08251652 19940531 US ABANDONED

child 08251652 19940531 US

parent continuation-of 08094382 19930721 US ABANDONED

child 08094382 19930721 US

parent continuation-of 07458754 19891211 US ABANDONED

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE

EP 87 08090 1987EP-87 08090 June 10, 1987
FR 88 401 121.4 1988FR-88 401 121.4 May 6, 1988

US-CL-CURRENT: 800/279, 435/252.3 , 435/320.1 , 435/419 , 435/69.2 , 514/12 , 530/350 , 536/23.2

ABSTRACT:

This invention relates to vectors, bacterial strains, and methods for the cloning and expression of a polypeptide having larvicidal activity. In particular, the invention relates to vectors, bacterial strains and methods for the cloning and expression of the N-terminal region of a polypeptide toxic against the larvae of Lepidoptera of the Noctuidae family, preferably against S. littoralis.

---------- KWIC ---------

Detail Description Paragraph - DETX (96):
[0180] (20) Adang et al, (1985) characterized full-length and **truncated plasmid clones of the crystal protein of Bacillus thuringiensis** subsp. kurstaki HD-73 and their toxicity to Manduca sexta. Gene 3: 289-300.

PGPUB-DOCUMENT-NUMBER: 20030106093

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030106093 A1

TITLE: Pesticidal proteins

PUBLICATION-DATE: June 5, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Narva, Kenneth E.	San Diego	CA	US	
Schnepf, H. Ernest	San Diego	CA	US	
Knuth, Mark	Poway	CA	US	
Pollard, Michael R.	Okemos	MI	US	
Cardineau, Guy A.	Poway	CA	US	
Schwab, George E.	Encinitas	CA	US	
Michaels, Tracy Ellis	Escondido	CA	US	
Lee, Stacey Finstad	San Diego	CA	US	
Burmeister, Paula	Ramona	CA	US	
Dojillo, Joanna	San Diego	CA	US	

APPL-NO: 10/ 099278

DATE FILED: March 15, 2002

RELATED-US-APPL-DATA:

child 10099278 A1 20020315

parent continuation-of 09378088 19990820 US GRANTED

parent-patent 6372480 US

child 09378088 19990820 US

parent continuation-in-part-of 08844188 19970418 US GRANTED

parent-patent 6127180 US

child 08844188 19970418 US

parent continuation-in-part-of 08633993 19960419 US GRANTED

parent-patent 6083499 US

US-CL-CURRENT: 800/279, 435/183 , 435/320.1 , 435/419 , 435/69.1 , 514/12 , 536/23.2

ABSTRACT:

The subject invention concerns new classes of pesticidally active proteins and the polynucleotide sequences which encode these proteins. More specifically, in preferred embodiments, pesticidal proteins of approximately 40-50 kDa and of approximately 10-15 kDa are used for controlling corn rootworms. Also, described are novel pesticidal isolates of Bacillus thuringiensis.

CROSS-REFERENCE TO A RELATED APPLICATION

[0001] This application is a continuation of application Ser. No. 09/378,088, filed Aug. 20, 1999, which is a continuation-in-part of application Ser. No. 08/844,188, filed Apr. 18, 1997, now U.S. Pat. No. 6,127,180; which is a continuation-in-part of Ser. No. 08/633,993, filed Apr. 19, 1996, now U.S. Pat. No. 6,083,499.

---------- KWIC ---------

Detail Description Paragraph - DETX (153):
[0176] (b) said toxin immunoreacts with an antibody to an approximately 40-50 kDa pesticidal **toxin, or a fragment thereof, from a Bacillus thuringiensis** isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554;

Detail Description Paragraph - DETX (158):
[0181] (g) said toxin immunoreacts with an antibody to an approximately 10-15 kDa pesticidal **toxin, or a fragment thereof, from a Bacillus thuringiensis** isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554;

PGPUB-DOCUMENT-NUMBER: 20030101482

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030101482 A1

TITLE: Compositions encoding lepidopteran-toxic polypeptides and methods of use

PUBLICATION-DATE: May 29, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Baum, James A.	Doylestown	PA	US	
Gilmer, Amy Jelen	Langhorne	PA	US	
Mettus, Anne-Marie Light	Feasterville	PA	US	

APPL-NO: 09/ 972175

DATE FILED: October 5, 2001

RELATED-US-APPL-DATA:

child 09972175 A1 20011005

parent division-of 09337635 19990621 US PATENTED

child 09337635 19990621 US

parent division-of 08980071 19971126 US PATENTED

child 08980071 19971126 US

parent continuation-in-part-of 08757536 19961127 US PATENTED

US-CL-CURRENT: 800/279, 435/184 , 435/320.1 , 435/410 , 536/23.7

ABSTRACT:

Disclosed are novel synthetically-modified B. thuringiensis nucleic acid segments encoding .delta.-endotoxins having insecticidal activity against lepidopteran insects. Also disclosed are synthetic crystal proteins encoded by these novel nucleic acid sequences. Methods of making and using these genes and proteins are disclosed as well as methods for the recombinant expression, and transformation of suitable host cells. Transformed host cells and transgenic plants expressing the modified endotoxin are also aspects of the invention. Also disclosed are methods for modifying, altering, and mutagenizing specific loop regions between the .alpha. helices in domain 1 of these crystal proteins, including Cry1C, to produce genetically-engineered recombinant cry* genes, and the proteins they encode which have improved insecticidal activity. In preferred embodiments, novel Cry1C* amino acid

segments and the modified cry1C* nucleic acid sequences which encode them are disclosed.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):
[0008] .delta.-endotoxins are a large collection of insecticidal proteins produced by B. thuringiensis. Over the past decade research on the structure and function of B. thuringiensis toxins has covered all of the major toxin categories, and while these toxins differ in specific structure and function, general similarities in the structure and function are assumed. Based on the accumulated knowledge of B. thuringiensis toxins, a generalized mode of action for B. thuringiensis toxins has been created and includes: ingestion by the insect, solubilization in the insect midgut (a combination stomach and small intestine), resistance to digestive enzymes sometimes with partial **digestion actually "activating" the toxin,** binding to the midgut cells, formation of a pore in the insect cells and the disruption of cellular homeostasis (English and Slatin, 1992).

Summary of Invention Paragraph - BSTX (70):
[0069] As a second illustrative embodiment, an alanine substitution for an arginine residue within or adjacent to the loop region between .alpha.-helices 4 and 5 produced a modified crystal protein with enhanced insecticidal activity (Cry1C-R148A). Although this substitution removes a potential trypsin-cleavage site within domain 1, trypsin **digestion of this modified crystal protein** revealed no difference in proteolytic stability from the native Cry1C protein.

Summary of Invention Paragraph - BSTX (159):
[0158] Other nucleic acid amplification procedures include transcription-based amplification systems (TAS) (Kwoh et al., 1989; PCT Intl. Pat. Appl. Publ. No. WO 88/10315, incorporated herein by reference in its entirety), including nucleic acid sequence based amplification (NASBA) and 3SR. In NASBA, the nucleic acids can be prepared for amplification by standard phenol/chloroform extraction, heat denaturation of a sample, treatment with lysis buffer and minispin columns for isolation of DNA and RNA or guanidinium chloride extraction of RNA. These amplification techniques involve annealing a primer which has crystal protein-specific sequences. Following polymerization, DNA/RNA hybrids are digested with RNase H while double stranded DNA molecules are heat denatured again. In either case the single stranded DNA is made fully double stranded by addition of second crystal protein-specific primer, followed by polymerization. The double stranded DNA molecules are then multiply transcribed by a polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNAs are reverse transcribed into double stranded DNA, and transcribed once against with a polymerase such as T7 or SP6. The resulting products, whether **truncated or complete, indicate crystal protein**-specific sequences.

Summary of Invention - Table CWU - BSTL (1):

1TABLE 1 REVISED B. THURINGIENSIS .delta.-ENDOTOXIN NOMENCLATURE.sup.A New Old GenBank Accession # Cry1Aa CryIA(a) M11250 Cry1Ab CryIA(b) M13898 Cry1Ac CryIA(c) M11068 Cry1Ad CryIA(d) M73250 Cry1Ae CryIA(e) M65252 Cry1Ba CryIB X06711 Cry1Bb ET5 L32020 Cry1Bc PEG5 Z46442 Cry1Bd CryE1 U70726 Cry1Ca CryIC X07518 Cry1Cb CryIC(b) M97880 Cry1Da CryID X54160 Cry1Db PrtB Z22511 Cry1Ea CryIE X53985 Cry1Eb CryIE(b) M73253 Cry1Fa CryIF M63897 Cry1Fb PrtD Z22512 Cry1Ga PrtA Z22510 Cry1Gb CryH2 U70725 Cry1Ha PrtC Z22513 Cry1Hb U35780 Cry1Ia CryV X62821 Cry1Ib CryV U07642 Cry1Ja ET4 L32019 Cry1Jb ET1 U31527 Cry1K U28801 Cry2Aa CryIIA M31738 Cry2Ab CryIIB M23724 Cry2Ac CryIIC X57252 Cry3A CryIIIA M22472 Cry3Ba CryIIIB X17123 Cry3Bb CryIIIB2 M89794 Cry3C CryIIID X59797 Cry4A CryIVA Y00423 Cry4B CryIVB X07423 Cry5Aa CryVA(a) L07025 Cry5Ab CryVA(b) L07026 Cry5B U19725 **Cry6A** **CryVIA** L07022 **Cry6B CryVIB** L07024 Cry7Aa CryIIIC M64478 Cry7Ab CryIIICb U04367 Cry8A CryIIIE U04364 Cry8B CryIIIG U04365 Cry8C CryIIIF U04366 Cry9A CryIG X58120 Cry9B CryIX X75019 Cry9C CryIH Z37527 Cry10A CryIVC M12662 Cry11A CryIVD M31737 Cry11B Jeg80 X86902 Cry12A CryVB L07027 Cry13A CryVC L07023 Cry14A CryVD U13955 Cry15A 34kDa M76442 Cry16A cbm71 X94146 Cry17A cbm71 X99478 Cry18A CryBP1 X99049 Cry19A Jeg65 Y08920 Cyt1Aa CytA X03182 Cyt1Ab CytM X98793 Cyt1B U37196 Cyt2A CytB Z14147 Cyt2B CytB U52043 .sup.aAdapted from: http://epunix.biols.susx.ac.uk/Home/Neil_Cri- ckmore/Bt/index.html

Detail Description Paragraph - DETX (11):

[0225] According to this invention, base substitutions are made in cry1C codons in order to change the particular codons encoding amino acids within or near the predicted loop regions between the .alpha.-helices of domain 1. As an illustrative embodiment, changes in three such amino acids within the loop region between .alpha.-helices 3 and 4 of domain 1 produced modified crystal proteins with enhanced insecticidal activity (Cry1C.499, Cry1C.563, Cry1C.579). As a second illustrative embodiment, an alanine substitution for an arginine residue within or adjacent to the loop region between .alpha.-helices 4 and 5 produced a modified crystal protein with enhanced insecticidal activity (Cry1C-R148A). Although this substitution removes a potential trypsin-cleavage site within domain 1, trypsin **digestion of this modified crystal protein** revealed no difference in proteolytic stability from the native Cry1C protein. Furthermore, the R180A substitution in Cry1C (Cry1C-R180A) also removes a potential trypsin cleavage site in domain 1, yet this substitution has no effect on insecticidal activity. Thus, the steps in the Cry1C protein mode-of-action impacted by these amino acid substitutions have not been determined nor is it obvious what substitutions need to be made to improve insecticidal activity.

US-PAT-NO: 6603063

DOCUMENT-IDENTIFIER: US 6603063 B1

TITLE: Plants and cells transformed with a nucleic acid from Bacillus thuringiensis strain KB59A4-6 encoding a novel SUP toxin

DATE-ISSUED: August 5, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Feitelson; Jerald S.	San Diego	CA	N/A	N/A
Schnepf; H. Ernest	San Diego	CA	N/A	N/A
Narva; Kenneth E.	San Diego	CA	N/A	N/A
Stockhoff; Brian A.	San Diego	CA	N/A	N/A
Schmeits; James	San Diego	CA	N/A	N/A
Loewer; David	San Diego	CA	N/A	N/A
Dullum; Charles Joseph	San Diego	CA	N/A	N/A
Muller-Cohn; Judy	Del Mar	CA	N/A	N/A
Stamp; Lisa	Solana Beach	CA	N/A	N/A
Morrill; George	El Cajon	CA	N/A	N/A
Finstad-Lee; Stacey	San Diego	CA	N/A	N/A

APPL-NO: 09/ 307106

DATE FILED: May 7, 1999

US-CL-CURRENT: 800/302, 435/252.3 , 435/418 , 536/23.71

ABSTRACT:

The specification discloses a nucleic acid from Bacillus thuringiensis strain KB59A4-6 that encodes a novel pesticidal SUP toxin and plants and microbial cells transformed with the nucleic acid.

12 Claims, 0 Drawing figures

Exemplary Claim Number: 10

---------- KWIC ---------

Brief Summary Text - BSTX (10):

More recently, new subspecies of B.t. have been identified, and genes responsible for active .delta.-endotoxin proteins have been isolated. Hofte and Whiteley classified B.t. crystal protein genes into four major classes (Hofte, H., H. R. Whiteley [1989] Microbiological Reviews 52(2):242-255). The classes were CryI (Lepidoptera-specific), CryII (Lepidoptera- and

Diptera-specific), CryIII (Coleoptera-specific), and CryIV (Diptera-specific). The discovery of strains specifically toxic to other pests has been reported. For example, CryV and **CryVI** have been proposed to designate a class of toxin genes that are nematode-specific.

Brief Summary Text - BSTX (22):

In one embodiment of the subject invention, Bacillus isolates can be cultivated under conditions resulting in high multiplication of the microbe. After treating the microbe to provide single-stranded genomic nucleic acid, the DNA can be contacted with the primers of the invention and subjected to PCR amplification. Characteristic **fragments of toxin**-encoding genes will be amplified by the procedure, thus identifying the presence of the toxin-encoding gene(s).

Brief Summary Text - BSTX (97):

It is apparent to a person skilled in this art that genes encoding active toxins can be identified and obtained through several means. The specific genes exemplified herein may be obtained from the isolates deposited at a culture depository as described above. These genes, or portions or variants thereof, may also be constructed synthetically, for example, by use of a gene synthesizer. Variations of genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as Bal31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which encode active fragments may be obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active **fragments of these toxins**.

Brief Summary Text - BSTX (98):

Equivalent toxins and/or genes encoding these equivalent toxins can be derived from Bacillus isolates and/or DNA libraries using the teachings provided herein. There are a number of methods for obtaining the pesticidal toxins of the instant invention. For example, antibodies to the pesticidal toxins disclosed and claimed herein can be used to identify and isolate toxins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the toxins which are most constant and most distinct from other Bacillus toxins. These antibodies can then be used to specifically identify equivalent toxins with the characteristic activity by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or Western blotting. Antibodies to the toxins disclosed herein, or to equivalent **toxins, or fragments of these toxins,** can readily be prepared using standard procedures in this art. The genes which encode these toxins can then be obtained from the microorganism.

Detailed Description Text - DETX (54):

The pMYC2610 HindIII **fragment containing the PS31F2 toxin** genes was isolated by restriction digestion, fractionation on a 0.7% agarose gel and purification from the gel matrix using the QiaexII kit (Qiagen Inc.; Valencia, Calif.). Gel purified insert DNA was then digested separately with restriction enzymes AluI,

MseI, or RsaI and fractionated on a 1% agarose gel. DNA fragments between 0.5 and 1.5 kb were excised from the gel and purified using the QiaexII kit. Recovered fragments were ligated into EcoRV digested pBluescriptII and transformed into E. coli XL10 Gold cells. Plasmid DNA was prepared from randomly chosen transformants, digested with NotI and ApaI to verify insert size and used as sequencing templates with primers homologous to plasmid vector sequences. Primer walking was used to complete the sequence. Sequencing reactions were performed using dRhodamine or BigDye Sequencing kit (ABI Prism/Perkin Elmer Applied Biosystems) and run on ABI 373 or 377 automated sequencers. Data was analyzed using Factura, Autoassembler (ABI Prism) and Gentics Computer Group (Madison, Wis.) programs. The MIS and WAR genes were found to be located next to one another in an apparent transcriptional operon. The WAR gene is 5' to the MIS gene, and the two genes are separated by 4 nucleotide bases.

Detailed Description Text - DETX (93):

In a preferred embodiment of the subject invention, plants will be transformed with genes wherein the codon usage has been optimized for plants. See, for example, U.S. Pat. No. 5,380,831. Also, advantageously, plants encoding a **truncated toxin** will be used. The **truncated toxin** typically will encode about 55% to about 80% of the full length toxin. Methods for creating synthetic Bacillus genes for use in plants are known in the art.

US-PAT-NO: H002074

DOCUMENT-IDENTIFIER: US H002074 H

TITLE: Fertile transgenic corn plants

DATE-ISSUED: July 1, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lundquist; Ronald C.	Minnetonka	MN	N/A	N/A
Walters; David A.	Bloomington	MN	N/A	N/A
Kirihara; Julie A.	Bloomington	MN	N/A	N/A

APPL-NO: 08/ 679001

DATE FILED: July 12, 1996

PARENT-CASE:

This is a division of application Ser. No. 08/618,749, filed Mar. 20, 1996, now U.S. Pat. No. 5,780,708, which is a division of application Ser. No. 08/285,488, filed Aug. 3, 1994, now U.S. Pat. No. 5,508,468, issued Apr. 16, 1996, which is a continuation of application Ser. No. 07/636,089, filed Dec. 28, 1990 now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 07/508,045, filed Apr. 11, 1990, now U.S. Pat. No. 5,484,956 issued Jan. 16, 1996, which in turn is a continuation-in-part of U.S. patent application Ser. No. 07/974,379, filed Nov. 10, 1992, now U.S. Pat. No. 5,538,877 issued Jun. 23, 1996 which in turn is a continuation of U.S. patent application Ser. No. 07/467,983, filed Jan. 22, 1990, now abandoned, all of which are incorporated by reference herein.

US-CL-CURRENT: 800/320, 536/24.1 , 800/278 , 800/301 , 800/302 , 800/303

ABSTRACT:

Fertile transgenic Zea mays (corn) plants which stably express recombinant DNA which is heritable are provided wherein said DNA preferably comprises a recombinant gene which encodes a seed storage protein, so that the amino acid profile of the corn is improved.

9 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Other Reference Publication - OREF (64):

M. J. Adang, et al., "Characterized Full Length and **Truncated Plasmid Clones of the Crystal Protein of Bacillus thuringiensis** subsp. kurstadki HD-73 and Their Toxicity to Maduca sexta", Gene, 36, 289-300, (1985).

US-PAT-NO: 6555655

DOCUMENT-IDENTIFIER: US 6555655 B1

TITLE: Coleopteran-toxic polypeptide compositions and insect-resistant transgenic plants

DATE-ISSUED: April 29, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rupar; Mark J.	Wilmington	DE	N/A	N/A
Donovan; William P.	Levittown	PA	N/A	N/A
Chu; Chih-Rei	Exton	PA	N/A	N/A
Pease; Elizabeth	Danville	PA	N/A	N/A
Tan; Yuping	Fremont	CA	N/A	N/A
Slaney; Annette C.	Burlington	NJ	N/A	N/A
Malvar; Thomas M.	Troy	MO	N/A	N/A
Baum; James A.	Webster Groves	MO	N/A	N/A

APPL-NO: 09/ 563269

DATE FILED: May 3, 2000

PARENT-CASE:

This application is based on U.S. Provisional Application No. 60/172,240, filed May 4, 1999, the entire contents of which are hereby incorporated by reference.

US-CL-CURRENT: 530/350, 536/23.71

ABSTRACT:

Disclosed are novel insecticidal polypeptides, and compositions comprising these polypeptides, peptide fragments thereof, and antibodies specific therefor. Also disclosed are vectors, transformed host cells, and transgenic plants that contain nucleic acid segments that encode the disclosed .delta.-endotoxin polypeptides. Also disclosed are methods of identifying related polypeptides and polynucleotides, methods of making and using transgenic cells comprising these polynucleotide sequences, as well as methods for controlling an insect population, such as Colorado potato beetle, southern corn rootworm and western corn rootworm, and for conferring to a plant resistance to a target insect species.

12 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

---------- KWIC ---------

Brief Summary Text - BSTX (7):

.delta.-endotoxins are used to control a wide range of leaf-eating caterpillars and beetles, as well as mosquitoes. These proteinaceous parasporal crystals, also referred to as insecticidal crystal proteins, crystal proteins, Bt inclusions, crystalline inclusions, inclusion bodies, and Bt toxins, are a large collection of insecticidal proteins produced by B. thuringiensis that are toxic upon ingestion by a susceptible insect host. Over the past decade research on the structure and function of B. thuringiensis toxins has covered all of the major toxin categories, and while these toxins differ in specific structure and function, general similarities in the structure and function are assumed. Based on the accumulated knowledge of B. thuringiensis toxins, a generalized mode of action for B. thuringiensis toxins has been created and includes: ingestion by the insect, solubilization in the insect midgut (a combination stomach and small intestine), resistance to digestive enzymes sometimes with partial **digestion actually "activating" the toxin,** binding to the midgut cells, formation of a pore in the insect cells and the disruption of cellular homeostasis (English and Slatin, 1992).

Detailed Description Paragraph Table - DETL (3):

TABLE 2 NOMENCLATURE OF KNOWN B. THURINGIENSIS .delta.-ENDOTOXINS.sup.A

New Old GenBank Accession # Cry1Aa1 CryIA(a) M11250 Cry1Aa2 CryIA(a) M10917 Cry1Aa3 CryIA(a) D00348 Cry1Aa4 CryIA(a) X13535 Cry1Aa5 CryIA(a) D175182 Cry1Aa6 CryIA(a) U43605 Cry1Aa7 AF081790 Cry1Aa8 126149 Cry1Aa9 AB026261 Cry1Ab1 CryIA(b) M13898 Cry1Ab2 CryIA(b) M12661 Cry1Ab3 CryIA(b) M15271 Cry1Ab4 CryIA(b) D00117 Cry1Ab5 CryIA(b) X04698 Cry1Ab6 CryIA(b) M37263 Cry1Ab7 CryIA(b) X13233 Cry1Ab8 CryIA(b) M16463 Cry1Ab9 CryIA(b) X54939 Cry1Ab10 CryIA(b) A29125 Cry1Ab11 I12419 Cry1Ab12 AF057670 Cry1Ac1 CryIA(c) M11068 Cry1Ac2 CryIA(c) M35524 Cry1Ac3 CryIA(c) X54159 Cry1Ac4 CryIA(c) M73249 Cry1Ac5 CryIA(c) M73248 Cry1Ac6 CryIA(c) U43606 Cry1Ac7 CryIA(c) U87793 Cry1Ac8 CryIA(c) U87397 Cry1Ac9 CryIA(c) U89872 Cry1Ac10 CryIA(c) AJ002514 Cry1Ac11 AJ130970 Cry1Ac12 I12418 Cry1Ad1 CryIA(d) M73250 Cry1Ad2 A27531 Cry1Ae1 CryIA(e) M65252 Cry1Af1 U82003 Cry1Ag1 AF081248 Cry1Ba1 CryIB X06711 Cry1Ba2 X95704 Cry1Bb1 ETS L32020 Cry1Bc1 CryIb(c) Z46442 Cry1Bd1 CryE1 U70726 Cry1Ca1 CryIC X07518 Cry1Ca2 CryIC X13620 Cry1Ca3 CryIC M73251 Cry1Ca4 CryIC A27642 Cry1Ca5 CryIC X96682 Cry1Ca6 CryIC X96683 Cry1Ca7 CryIC X96684 Cry1Cb1 CryIC(b) M97880 Cry1Da1 CryID X54160 Cry1Da2 176415 Cry1Db1 PrtB Z22511 Cry1Ea1 CryIE X53985 Cry1Ea2 CryIE X56144 Cry1Ea3 CryIE M73252 Cry1Ea4 U94323 Cry1Ea5 A15535 Cry1Eb1 CryIE(b) M73253 Cry1Fa1 CryIF M63897 Cry1Fa2 CryIF M63897 Cry1Fb1 PrtD Z22512 Cry1Fb2 Z22512 Cry1Fb3 AF062350 Cry1Fb4 173895 Cry1Ga1 PrtA Z22510 Cry1Gb1 CryIM Y09326 Cry1Gb1 CryH2 U70725 Cry1Ha1 PrtC Z22513 Cry1Hb1 U35780 Cry1Ia1 CryV X62821 Cry1Ia2 CryV M98544 Cry1Ia3 CryV L36338 Cry1Ia4 CryV L49391 Cry1Ia5 CryV Y08920 Cry1Ia6 AF076953 Cry1Ib1 CryV U07642 Cry1Ic1 AF056933 Cry1Ja1 ET4 L32019 Cry1Jbl ET1 U31527 Cry1Jc1 AF056933 Cry1Ka1 U28801 Cry2Aa1 CryIIA M31738 Cry2Aa2 CryIIA M23723 Cry2Aa3 D86084 Cry2Aa4 AF047038 Cry2Aa5 AJ132464 Cry2Aa6 AJ1324635 Cry2Aa7 AJ132463 Cry2Ab1 CryIIB M23724 Cry2Ab2 CryIIB X55416 Cry2Ac1 CryIIC X57252 Cry3Aa1 CryIIIA M22472 Cry3Aa2 CryIIIA

J02978 Cry3Aa3 CryIIIA Y00420 Cry3Aa4 CryIIIA M30503 Cry3Aa5 CryIIIA M37207 Cry3Aa6 CryIIIA U10985 Cry3Aa7 AJ237900 Cry3Ba1 CryIIIB X17123 Cry3Ba2 CryIIIB A07234 Cry3Bb1 CryIIIB2 M89794 Cry3Bb2 CryIIIC(b) U31633 Cry3Bb3 I15475 Cry3Ca1 CryIIID X59797 Cry4Aa1 CryIVA Y00423 Cry4Aa2 CryIVA D00248 Cry4Ba1 CryIVB X07423 Cry4Ba2 CryIVB X07082 Cry4Ba3 CryIVB M20242 Cry4Ba4 CryIVB D00247 Cry5Aa1 CryVA(a) L07025 Cry5Ab1 CryVA(b) L07026 Cry5Ac1 I34543 Cry5Ba1 PS86Q3 U19725 Cry6Aa1 **CryVIA** L07022 Cry6Ba1 **CryVIB** L07024 Cry7Aa1 CryIIIC M64478 Cry7Ab1 CryIIICb U04367 Cry7Ab2 U04368 Cry8Aa1 CryIIIE U04364 Cry8Ba1 U04365 Cry8Ca1 U04366 Cry8Ba1 CryIIIG U04365 Cry8Ca1 CryIIIF U04366 Cry9Aa1 CryIG X58120 Cry9Aa2 CryIG X58534 Cry9Ba1 CryIX X75019 Cry9Ca1 CryIH Z37527 Cry9Da1 N141 D85560 Cry9Da2 AF042733 Cry9Ea1 Cry10Aa1 CryIVC M12662 Cry10Aa2 E00614 Cry11Aa1 CryIVD M31737 Cry11Aa2 CryIVD M22860 Cry11Ba1 Jeg80 X86902 Cry11Bb1 AF017416 Cry12Aa1 CryVB L07027 Cry13Aa1 CryVC L07023 Cry14Aa1 CryVD U13955 Cry15Aa1 34kDa M76442 Cry16Aa1 cbm71 X94146 Cry17Aa1 cbm71 X99478 Cry18Aa1 CryBP1 X99049 Cry19Aa1 Jeg65 Y08920 Cry20Aa1 U82518 Cry21Aa1 I32932 Cry22Aa1 I34547 Cry23Aa1 AF03048 Cry24Aa1 U88188 Cry25Aa1 U88188 Cry26Aa1 AF122897 Cry27Aa1 AB023293 Cry28Aa1 AF132928 Cyt1Aa1 CytA X03182 Cyt1Aa2 CytA X04338 Cyt1Aa3 CytA Y00135 Cyt1Aa4 CytA M35968 Cyt1Ab1 CytM X98793 Cyt1BA1 U37196 Cyt2Aa1 CytB Z14147 Cyt2Ba1 "CytB" U52043 Cyt2Ba2 "CytB" AF020789 Cyt2Ba3 "CytB" AF022884 Cyt2Ba4 "CytB" AF022885 Cyt2Ba5 "CytB" AF022886 Cyt2Ba6 AF034926 Cyt2Bb1 U82519 Cyt2Bb1 U82519

.sup.a Adapted from: Crickmore, N. et al. Microbiol. and Mol. Bio. Rev. (1998) Vol. 62: 807-813